Effects of melatonin and zinc on glycemic control in type 2 diabetic patients poorly controlled with metformin

Saad A. Hussain, *MSc, PhD,* ***Haitham M. Khadim,*** *MSc,* ***Ban H. Khalaf,*** *MSc, PhD,*
Sajida H. Ismail, *MSc, PhD,* ***Khalid I. Hussein,*** *MBChB, FABM,* ***Ahmed S. Sahib,*** *MSc.*

ABSTRACT

Objectives: This project was designed to evaluate the effects of melatonin and zinc on the glycemic control in type 2 diabetes mellitus (T2DM) patients with inadequate response to the oral hypoglycemic agent metformin.

Methods: A placebo controlled, double-blind clinical trial was performed at the Specialized Center for Endocrinology and Diabetes, Al-Rusafa Directorate of Health, Baghdad, Iraq during the period from February to July 2005, in which 46 type 2 diabetic patients were selected and allocated into 3 groups, these groups were treated with single daily oral doses of both 10 mg melatonin and 50 mg zinc acetate alone; 10 mg melatonin and 50 mg zinc acetate in addition to the regularly used metformin or placebo, given at bed time for 90 days. We measured the fasting plasma glucose (FPG), glycated hemoglobin (HbA1C) and serum C-peptide before starting the treatment (zero time) and after 30 and 90 days of treatment. We also performed post-prandial glucose excursion test (PPGE) for selected patients from the second and third groups before starting the treatment and after 90 days.

Results: Daily administration of melatonin and zinc improved the impaired fasting and post-prandial glycemic control and decreased the level of glycated hemoglobin; addition of this treatment regimen in combination with metformin improved the tissue responses to this oral hypoglycemic agent.

Conclusion: The combination of melatonin and zinc acetate, when used alone or in combination with metformin improves fasting and post-prandial glycemic control in T2DM patients.

Saudi Med J 2006; Vol. 27 (10): 1483-1488

Diabetes mellitus (DM) is a state of chronic hyperglycemia (excess circulating glucose), classically associated with symptoms of excessive thirst, increased urine volume, and, if severe enough, weight loss.[1] The pathophysiologic basis for type 2 diabetes rests in defects in insulin secretion (hypo and hyperinsulinemia), decreased tissue responsiveness to insulin (insulin resistance/sensitivity), abnormal hepatic glucose production and impaired glucose metabolism.[2] As long as there is an adequate compensatory insulin secretion, normal glucose tolerance is maintained. Impaired insulin secretion results in the pathogenesis of glucose intolerance. Pancreatic islet function (ß-cells) in type 2 diabetes is characterized by decreased responsiveness to a glucose challenge, and loss of the "first-phase" of insulin release after food uptake very early in the development of type 2 diabetes.[3] In the uncontrolled

From the Department of Pharmacology and Toxicology (Hussain, Ismail), College of Pharmacy, University of Baghdad, Department of Pharmacology and Therapeutics (Khalaf), College of Pharmacy, Al-Mustansrya University, Specialized Center for Endocrinology and Diabetes (Hussein), Al-Rusafa Directorate of Health, Baghdad, Department of Basic Sciences (Khadim), College of Medicine, University of Diyala, Baquba, and the Department of Pharmacy (Sahib), Directorate of Health, Diyala, *Iraq*.

Received 12th February 2006. Accepted for publication in final form 8th July 2006.

Address correspondence and reprint request to: Dr. Saad A. Hussain, Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad, Baghdad, *Iraq*. Tel. +964 (25) 532418. E-mail: saad_alzaidi@yahoo.com

type 2 diabetic patient, periods of acute hyperglycemia occur following the ingestion of food. This mealtime glucose hyperglycemia (or mealtime glucose excursions "Spikes") makes a major contribution to the overall poor glycemic control. Long periods of hyperglycemia occur around mealtimes lead to elevated mean levels of glucose over any 24 hours period. Moreover, rapid and excessive rises in blood glucose have been implicated in the progression of insulin deficiency, particularly through pancreatic ß-cell over stimulation, further tissue damage and insulin resistance. This had obvious implications for the patient and it is possible that these episodes make a significant contribution to the disease process and the development of secondary complications.[4] Evidence that oxidative stress is present in diabetes originates from the frequent observation that both reactive oxygen species (ROS) and antioxidants are increased. The later is logically rather seen in early stage of diabetes and should be interpreted as a tentative compensation of cells against increasing oxidative stress.[5,6] It has been demonstrated that melatonin show a marked protective effect against oxidative stress and severity of diabetes induced by streptozocin in rats, this confirm the powerful antioxidant action of this pineal indole, and the importance of the severity of oxidative stress to maintain hyperglycemia and protein glycosylation, 2 pathogenetic cornerstones indicative of diabetic complications.[7] Hassan et al[8] reported that the regular intake of 3 mg/day melatonin by diabetic patients on diet therapy, lead to a time dependent improvement in glycemic control and other related diabetic complications.[8] Accordingly, this study was designed to evaluate the possible role of using melatonin in regular daily pharmacological doses (10 mg/day) in association with zinc acetate in required daily levels, in the improvement of glycemic control of type 2 DM in patients treated with metformin, but with poor control.

Methods. This study was performed on 46 patients with type 2 DM at the Specialized Center for Endocrinology and Diabetes, Al-Rusafa Directorate of Health, Baghdad, Iraq during the period from February to July 2005. The Scientific Committee approved the study protocol. We included 25 males and 21 females with an age range of 40-64 years (49.1 ± 6.0), and a disease duration of 4.2 ± 3.1 years. All selected patients had no other marked of pathological disorders such as hypertension and ischemic heart diseases (IHD) as revealed by the clinical investigation. Thirty-three patients were treated previously with maximal dose of metformin (Menarini International, Italy) (2,550 mg/day) 3 times daily, and kept on dietary regulation but with poor glycemic control, as evidenced of abnormal values of fasting plasma glucose and glycated hemoglobin. These patients were carefully evaluated while they were on their treatment program for DM control for 2 weeks before being included in 3 groups: Group A comprise 15 patients (8 males and 7 females) treated with placebo in capsule form in addition to being given the oral hypoglycemic agent (metformin 2,550 mg/day) and under dietary control for 3 months. Group B comprise 18 patients (11 males and 7 females) treated with 10 mg melatonin (Rupal Chemicals Ltd, India) and 50 mg of zinc acetate (Fluka Chemie, Switzerland), given as single daily doses in a capsule form, in addition to the oral hypoglycemic agent (metformin 2,550 mg/day) and under dietary control for 3 months. Group C included the remaining 13 DM patients (7 males and 6 females) that were newly diagnosed and maintained for 2 weeks on dietary control program only; they were treated with 10 mg melatonin and 50 mg zinc acetate as single daily doses in capsule form in addition to the dietary control program for 3 months. Seventeen healthy subjects (9 males and 8 females) in the same age range as that of patients were selected and served as controls. All patients were selected according to the following criteria: they did not have other associated chronic diseases such as liver or kidney disorders and no cardiovascular complications. Patients who were pregnant or breast-feeding were excluded. They were not on insulin therapy or on antioxidant drugs including aspirin, or on any associated drugs. After 12 hours fasting, blood samples (10 ml) were collected from all subjects via venous puncture before starting drug treatment (zero time sample) and then after 30 days and 90 days of treatment to follow the changes in the studied parameters. Blood samples were collected in citrate-containing tube and centrifuged at 3,000 rpm for 10 min at 4^0C; after centrifugation and isolation of cellular fraction, the plasma fraction was stored frozen until the analysis was performed. Plasma glucose level was evaluated using a ready made kit for this purpose, according to the method of Barham and Trindoer,[9] glycated hemoglobin was analyzed utilizing the principles of ion exchange high performance liquid chromatography (HPLC) for the automatic and accurate separation of glycated hemoglobin (HbA_{IC}).[10] C-peptide level was determined by an enzymatic immunoassay.[11] After 12 hours fasting, 7 patients belong to group A and 6 patients belong to group B were involved in performed post-prandial glucose excursion test (PPGE) test before and after initiating treatment with melatonin and zinc; where fasting blood sample was taken, then a standard solid meal (530 kcal) that contain 59% carbohydrate, 30%

fat and 20% protein was given. Blood samples were drawn after 30, 60, 120, 180 and 240 min and plasma glucose level was evaluated as indicated later. Area under the curve (AUC_{0-4} hours) was calculated for the plot of plasma glucose versus time.[12]

Student's t–test and analysis of variance test were used to examine the degree of significance, and the probability value less than 0.05 was considered significant.

Results. The data presented in **Table 1** indicated that all selected groups of DM patients had significantly higher levels of fasting plasma glucose (FPG) and HbA1C, associated with significantly lower C-peptide levels compared to controls, indicating poor glycemic control by full dose of metformin and/or dietary control. In group A patients, placebo formula produced significant decrease in FPG levels (9%) after 90 days, while HbA1C significantly reduced after 30 days of treatment (14%) and remain as such until the end of treatment period. However, C-peptide levels did not show any significant changes during the treatment period. Treatment with a combination of melatonin and Zinc acetate, in addition to metformin (group B) resulted in significant reduction ($p<0.01$) in FPG (25%) and HbA_{1C} (17%) levels after 30 days of treatment. Glycated hemoglobin levels showed further significant reduction after 90 days (26%). In this group, while no significant changes were observed in C-peptide levels during the treatment period (**Table 1**). Concerning group C patients, treatment with melatonin and zinc only, in addition to dietary control, resulted in 23% decrease in FPG levels after 90 days, which is significantly different compared to baseline value ($p<0.01$). Such type of treatment also resulted in 15% and 29% reduction in HbA1C levels after 30 and 90 days respectively, and found significantly different compared to baseline value ($p<0.01$). No change in C-peptide level was observed during the treatment period ($p>0.05$) (**Table 1**). When the effects of the 3 different approaches of treatment on the FPG levels were compared, adjunct use of melatonin and zinc with metformin produces the highest reduction in FPG levels (group B) compared to others ($p<0.05$). The effect of melatonin and zinc, when used alone (group C) or in combination with metformin (group B), produced comparable effects on HbA1C levels after 90 days (28.7% and 26.2%), which are significantly higher than the placebo effect (group A) ($p<0.01$). **Table 1** revealed no significant differences between the effects of the tested formula on C-peptide levels compared to placebo in all circumstances. **Table 2** showed that area under the curve that represents the relationship between plasma

Table 1 - Effects of treatment with 10 mg melatonin and 50 mg zinc acetate on FPG, HbA1c and C-peptide levels in type 2 diabetic patients poorly controlled with metformin.

Patients groups/durations	FPG (mmol/l)	HbA1c%	C-peptide (pmol/l)
Control (n=17)	5.2 ± 0.131	5.0 ± 0.216	419.6 ± 9.367
Group A (n=15)			
Placebo + Metformin			
Baseline	10.2 ± 0.7[a*]	8.86 ± 0.35[a*]	172.6 ± 44.62[a*]
30 days	9.34 ± 0.84[a]	7.59 ± 0.5[b]	190.4 ± 36.86[a]
90 days	9.3 ± 1.027[b]	7.80 ± 0.5[b]	177.3 ± 29.75[a]
Group B (n=18)			
Melatonin+Zinc+Metformin			
Baseline	12.7 ± 0.86[a*]	7.73 ± 0.45[a*]	319.3 ± 56.19[a*]
30 days	9.56 ± 1.27[b]	6.41 ± 0.36[b]	325.6 ± 65.99[a]
90 days	7.83 ± 0.54[b]	5.71 ± 0.32[c†]	305.3 ± 50.14[a]
Group C (n=13)			
Melatonin + Zinc			
Baseline	8.61 ± 0.61[a*]	7.60 ± 0.47[a*]	271.5 ± 62.6[a*]
30 days	8.13 ± 0.55[a]	6.47 ± 0.57[b]	294.1 ± 73.94[a]
90 days	6.63 ± 0.47[b]	5.41 ± 0.35[c†]	330.6 ± 81.05[a]

Results were presented as mean ± standard error. Results with non-identical superscripts (a, b, c) among the same group were considered significantly different ($p<0.01$). *Significant difference from control, $p<0.05$. †Significantly lower values with respect to group A after 90 days ($p<0.05$). FPG- fasting plasma glucose, HbA1c - glycated hemoglobin

Table 2 - Effects of daily treatment with 10 mg melatonin and 50 mg zinc acetate for 60 days on the area under the curve (AUC_{0-4}) of PPGE in type 2 diabetic patients poorly controlled with metformin.

Patients groups/duration	AUC_{0-4} (min. mmol/l)
Control n = 12	32.32 ± 0.44
Group A (Placebo + Met) n = 6	
Baseline	97.8 ± 3.20[a*]
60 days	98.7 ± 0.82[a]
Group B (Mel + Zinc + Met) n =7	
Baseline	80.31 ± 3.07[a*]
60 days	58.04 ± 2.28[b]

Results with non-identical superscripts (a,b) within the same group were considered significantly different ($p<0.01$). *significantly different compared to controls ($p<0.05$). Results were presented as mean ± S.E. Met - metformin, Mel - melatonin, PPGE - post-prandial glucose excursion

glucose levels and time, after oral meal challenge, were significantly higher in both groups (202% and 148%) compared to control. Meanwhile, there were no significant differences between them ($p>0.05$) before starting the treatment. After 60 days of treatment with melatonin and zinc (group B) and placebo (group A) in addition to metformin, **Table 2** clearly showed that AUC_{0-4} in group B was significantly lower than that resulted due to treatment with placebo in group A (41%). The percent change in AUC with respect to baseline in group B patients was significantly higher (27.7%, $p<0.01$) compared to that in group A patients ($p>0.05$).

Discussion. In the present study, treatment of type 2 diabetic patients, poorly controlled with metformin, with melatonin and zinc improves glycemic control through decreasing FPG and HbA1C levels, results that are comparable to those when they are used alone for glycemic control. The pineal gland, through its secretory product melatonin, is believed to take part in the energy balance control of hibernating mammals.[13] Meanwhile, the response to a variety of stimuli (metabolic, hormonal and neural) and the pattern of insulin secretion by the pancreatic ß-cells vary according to the time of the day.[14] In humans, the response of pancreatic ß-cells to glucose challenge is more intense in the morning hours and decline in the evening, provided the same amount of glucose is administered.[15] The relationship between pineal gland, melatonin and the regulation of carbohydrate metabolism has been suggested in both humans and rodents. Pinealectomy induces a decrease in hepatic and muscular glycogenesis, and an increase in blood pyruvate concentration in rats.[16] Moreover, pinealectomy in rats was followed by an increase in blood sugar levels compared to those with intact pineal gland controls.[17] Conversely, infusion of pineal extract lead to hypoglycemia and increased both, glucose tolerance and muscular glycogenesis after glucose loading.[16] Lima et al[18] showed that, in isolated rat's adipocytes, melatonin elicited an enhancement in cell sensitivity to insulin (a leftward shift in the insulin-stimulated dose-response curve). Furthermore, it is well known that both, humans and rats show diurnal fluctuation in the oral and intravenous glucose tolerance tests,[19,20,21] and a clear diurnal variation in peripheral sensitivity to insulin.[22] Because melatonin seems to be the main putative timing molecule in several physiological systems,[23,24] and plays an important role in the regulation of peripheral actions of insulin,[25] its secretion seems critical for the daily regulation of ß-cells ability to respond to elevated glucose levels.[18] The presumed action of melatonin, as a regulator of ß-cells responsiveness to glucose, may be exerted by both direct and indirect mechanisms.[26] Indirect effects of pineal activity over insulin target tissue cannot be discarded; the pineal gland can modify the functions of the suprachiasmatic nuclei and the well established modulator of carbohydrate metabolism.[27] Melatonin may interfere with GLUT-4 synthesis regulation, [28] an insulin-dependent activity in the plasma membrane, which regulates translocation of glucose.[18] The results presented in this study showed no significant changes in C-peptide levels due to treatment with melatonin compared to controls. This gives an indication about the predominance of the indirect way as a mechanism for the explanation of improving glycemic control. However, the direct effect on insulin secretion by ß-cells may require longer duration of treatment than that followed in this study. Melatonin has found to elevate plasma zinc levels and normalizes zinc balance in senescent animals.[29] There is a signaling system, found to be regulated by zinc, stimulates glucose transport through a post-insulin receptor mechanism.[30] On the other hand, zinc may be involved in the pathway of insulin signaling through the inhibition of certain membrane-associated tyrosine phosphatase activity, which is known to antagonize insulin effect at receptor level.[31,32] Zinc also stimulates both, membrane localization and activity of certain isoform of protein kinase-C, appeared to be important in insulin signaling.[33,34] All the indicated mechanisms for the role of zinc supported the multiple site involvement in insulin signaling.

Measurement of HbA1C levels, which represent the extent of glycosylation of hemoglobin, can be used as

a marker for the long-term effects of hyperglycemia, reveal that therapeutic use of melatonin and zinc significantly lowers Hb glycation. In diabetes, hyperglycemia derives non-enzymatic glycation and oxidation of proteins and lipids, which consequently enhances the formation of advanced glycosylation end products (AGEs) that are potentially involved in atherogenesis and diabetic vascular disorders.[35] Melatonin has been proven effective in glycemic control and reduction of protein glycosylation in experimentally-induced rats with DM.[36] Moreover, zinc complexes produced significant reduction in HbA1C levels in diabetic mice,[37] in addition to the proven positive relationship between HbA1C levels and the urinary loss of zinc in diabetic humans.[38] The data presented in this study are consistent with those indicated previously (**Table 1**).

Tables 1 and 2 clearly showed that treatment of type 2 diabetic patients with melatonin and zinc acetate significantly improves fasting and post-prandial glycemic control with no effect on C-peptide levels. This observation is not consistent with that obtained by Stewart et al,[39] which showed that consumption of zinc with drinking water increases plasma C-peptide levels. On the other hand, Sahib[40] showed that treatment of type 2 diabetic patients with zinc sulphate significantly decreases plasma C-peptide levels. In this study, no significant changes in C-peptide levels were observed, so, suggesting that melatonin and zinc decrease blood glucose in type 2 diabetic patients by a mechanism that not involve enhancement of insulin secretion.

In conclusion, melatonin and zinc, when used alone or adjunct to metformin, improve glycemic control in type 2 diabetic patients through a mechanism not related to increase insulin secretion.

Acknowledgment. We would like to thank the College of Pharmacy, University of Baghdad and the Specialized Center for Diabetes and Endocrinology, Baghdad, Iraq for supporting this project.

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